

## PIT-CLOSING MEMBRANE IN OPHIOGLOSSACEAE

GERTRUDE WRIGHT

(WITH PLATES XI, XII AND SIX FIGURES)

The members of the Ophioglossaceae, an isolated family of uncertain origin, are forms with a few large leaves, simple to compound, and short, slow growing, underground stems, vertical, oblique, or horizontal in position, with crowded fleshy roots. The leaves, which are divided into sterile and fertile lobes, bear on the latter homosporous sporangia.

Of the three widely distributed genera, *Helminthostachys*, a monotypic genus, is the most restricted, occurring throughout tropical Asia to North Australia and New Caledonia. *Ophioglossum* is represented by about 30 species growing under various conditions of moisture and shade in the temperate and tropical zones of both the Eastern and Western hemispheres. *Botrychium*, with nearly as many species, is world wide in its distribution, but is confined chiefly to the temperate regions.

The forms considered in this paper are *Helminthostachys zeylanica*, *Ophioglossum vulgatum*, the only species of the genus native to Canada, and *Botrychium obliquum*, one of the 6 or 8 forms found in Ontario.

The rhizome of *Ophioglossum vulgatum* consists of a large, starch-filled cortex surrounding a siphonostele of endarch bundles of primary wood. This cylinder may be broken by leaf gaps, often so prolonged as to overlap, producing a circle of bundles. Fig. 1 shows several such bundles, one, beside an outgoing root, starting on its way through the cortex to the petiole. There is no endodermis in the mature plant, and the pith is directly continuous with the cortex through the large leaf gaps.

*Helminthostachys*, whose rhizome is horizontal and dorsiventral, presents a slightly different appearance in cross-section. Fig. 2 shows its broad woody cylinder solid on the lower side, broken



on the upper right by a relatively small leaf gap beside an outgoing leaf trace. The wood is entirely primary, with groups of parenchyma scattered throughout it. The mesarch structure of the bundles is not evident here, but may be demonstrated by means of longitudinal sections. The large-celled, winding endodermis is, unfortunately, too faintly stained to show clearly in the figure. According to FARMER and FREEMAN (4), there is in this form cork formation confined to the upper surface and originating at the bases of the cast-off leaves.

The most extraordinary member of the group in regard to its wood structure, however, is undoubtedly *Botrychium*. In this form there occurs a well developed cylinder of secondary wood, as well as a definite cork layer. The stem shown in transverse section (fig. 3) illustrates this. The woody cylinder surrounding a rather large starchy pith is solid with the exception of small leaf gaps, one of which appears in the lower part of the figure to the left of a horizontal root. The wood, which is composed of tracheids of irregular size, is traversed by numerous uniseriate medullary rays of slightly radially elongated parenchyma. The few and inconspicuous primary bundles are endarch. The pericycle consists of several rows of parenchyma, and is surrounded by an endodermis, frequently multiple. A rather large cortex, also utilized in the storage of starch, is bounded by cork which is visible in the upper right-hand corner of the figure.

The roots of the three genera show no secondary wood of any account. BOODLE (2) has described the addition of a few tracheids at the base of the old roots of *Ophioglossum vulgatum* and *Botrychium Lunaria*, but the later formed parts show only typically primary bundles, in the case of the former genus monarch in structure, and in the latter triarch or tetrarch (figs. 4, 5). The hexarch stele of the *Helminthostachys* root also shows only primary arrangement (fig. 6).

The character of the wood elements themselves in the three genera differs almost as much as their arrangement. Fig. 7 shows the elements in the metaxylem of the root of *O. vulgatum*, stained with Haidenhain's iron-haematoxylin and safranin. They do not differ from those of the stem, hence they represent the general



condition, pitting of the bordered scalariform type. With this stain the primary wall shows broad and black through the secondary, dividing the narrow red borders of adjacent pits. This is most apparent in the upper half of the tracheid to the left, where the scalariform openings are uniseriate, extending from side to side of the tracheid. In the lower half of the tracheid the primary wall has not been cut. The pit borders are more or less clear, also, about the middle of the tracheid to the right where the pits are small, oval, and biseriate. A combination of silver nitrate solution and ammonia, used with a counter stain of methylene blue, demarked these borders most clearly, but, unfortunately, did not lend itself to photography.

On the other hand, the metaxylem of *Helminthostachys* and the metaxylem and secondary wood of *Botrychium* exhibit a much greater differentiation. The tracheids, as seen in longitudinal section, are irregular and frequently nodular in appearance, with pitting distributed equally on their radial and tangential walls. The section illustrated in fig. 14 is from the rhizome of *B. obliquum*, cut tangentially and stained with haematoxylin and safranin. The tracheids are irregular in size and position, and interspersed with uniseriate medullary rays. The central tracheid shows the typical pitting of the secondary wall. The uniseriate and biseriate pits are large, round to oval in shape, with a centrally placed round pore. The small shaded area surrounding the pore is lignified.<sup>1</sup> In the tracheids to right and left is depicted a feature characteristic of both *Botrychium* and *Helminthostachys*, a tertiary wall of lignin. About the center of the tracheid to the left this layer appears as reticulately arranged bars lying over the pitted secondary wall. Above the center the plane of section is lower, exposing only the secondary wall; below the center it is through the lumen of the tracheid, and consequently the tertiary layer is seen in section. In the tracheid to the right, both the tertiary and secondary walls have been cut only in section. Fig. 15, also from *B. obliquum*, gives a sectional view of the pits with their overlaid scalariform. The pit cavities are approximately twice as

<sup>1</sup> In all the text figures lignification has been indicated by means of shading, and a different focus or an obscure feature by dotted lines.



long as broad, and rounded at the ends. The spools between, forming their borders, show a fairly thick, secondary, unligified wall, ridged in most cases by one to two lignified (shaded) bars. The areas between the pits are small, and the primary wall which traverses them has, frequently, at the edges of the pits, thickenings

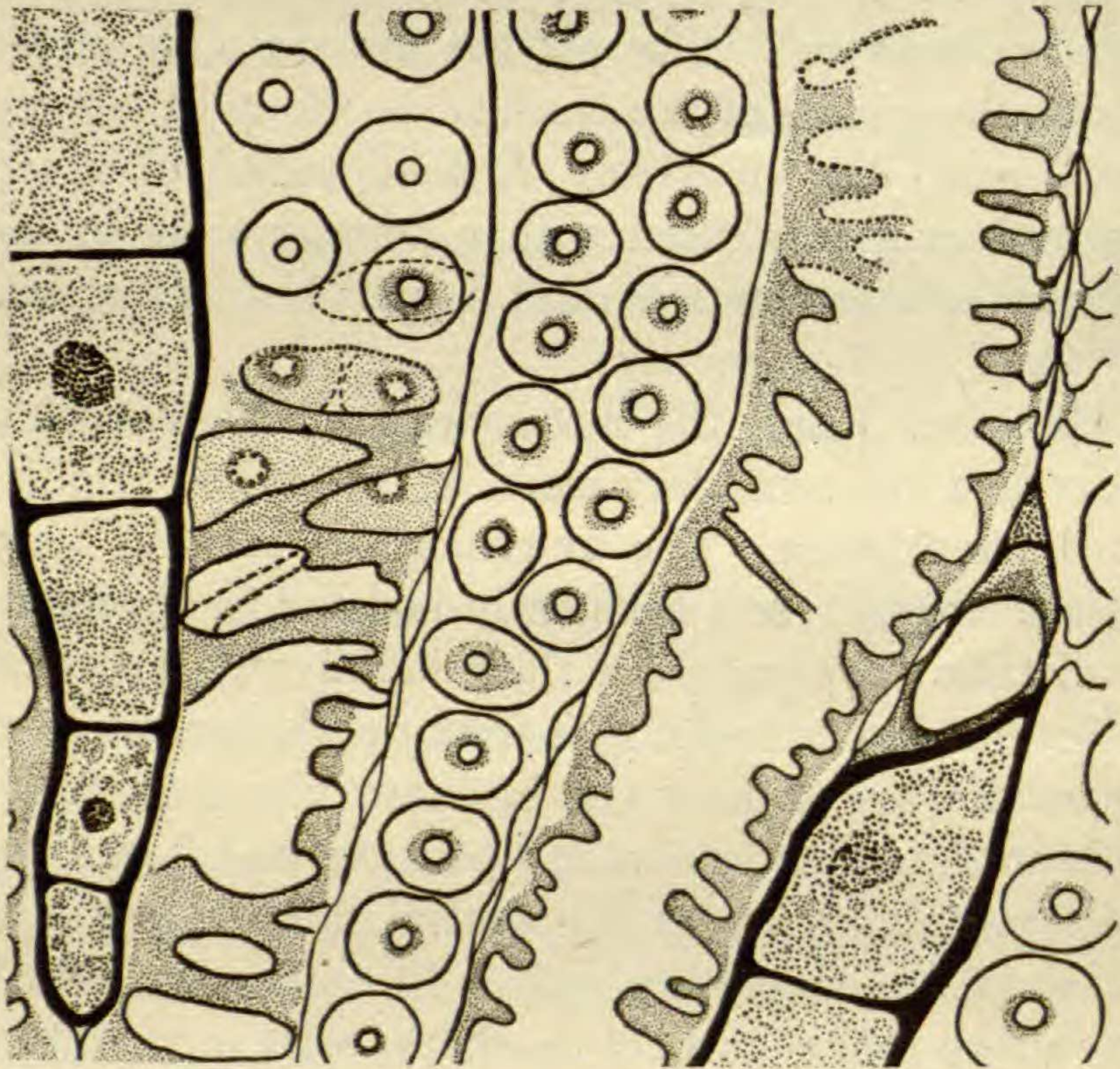


FIG. 14.—*Botrychium obliquum*: tangential section of the rhizome showing pitting;  $\times 600$ .

similar to bars of *Sanio*. These are shown on the last four spools toward the top of the figure.

Fig. 10 shows the stem wood of *Helminthostachys* to be fairly similar to that of *Botrychium*, as seen in figs. 14 and 15. To the right of the center the walls of two adjacent tracheids have been cut in section. The left-hand wall is composed of only the secondary layer, which is characteristically thinner than in *Botrychium*, that to the right, of tertiary bars as well. The reticulate arrangement of these bars may be seen in the tracheid to the left of the center. The first-formed elements of the metaxylem of both *Helminthostachys* and *Botrychium* show less of a tertiary layer than the later



formed ones figured here. The scalariform bars in the former are fine and rather far apart, in the latter broader and joined in such a way as to produce the reticulate effect of fig. 10.

In both *Botrychium* and *Helminthostachys* the tracheids of the root wood, although slightly smaller and more regular than those of the stem, resemble these very closely. There is, perhaps, a greater amount of open scalariform tertiary thickening than in the stem and less of the broad, close formation. The petiole wood of both forms is also a likeness in miniature of that of the stem, particularly of the first-formed elements of the primary metaxylem of the latter. Frequently, however, the pit pores in *Helminthostachys* petiole are long and oblique rather than round.

The presence or absence of a pit-closing membrane in the Ophioglossaceae, as in all the vascular cryptogams, has been a matter of dispute. RUSROW (7), in illustrating his article of 1872, expressed the prevailing view of the anatomists of his time with regard to the vascular cryptogams in general, when he showed no membrane in the pits of either the side or the end walls of *Botrychium*. It was in the following year that SANIO, working with *Pinus sylvestris*, demonstrated beyond a doubt the presence, in the mature condition in that form, not only of a membrane but also of a torus. From that time the pendulum of opinion began to swing in the opposite direction. In response to the stimulus of SANIO'S discovery, evidence has steadily accumulated that the membrane in the vascular cryptogams remains in the pits of the mature wood, not only in the side walls of the elements but, with few exceptions, in the end walls as well. In 1908, however, this view was challenged by GWYNNE-VAUGHAN (5). In returning to the idea of the earliest investigators, that the membrane disappears through resorption in the mature wood, the author distinguishes two types of ferns, represented by *Pteris* and *Osmunda* respectively. Ferns of the *Pteris* type, he claims, lose their limiting membrane only from the pit cavities, while those of the *Osmunda* type lose it also from between the walls of adjacent tracheids in the region between the pits. GWYNNE-VAUGHAN describes a further modification of this type which, however, need not be discussed here, as he classes the Ophioglossaceae with ferns of the *Pteris*



type. As far as the longitudinal walls are concerned, the opposite view, that of the persistence of the membrane in the pits, was upheld by HALFT (6) for "all the vascular cryptogams." He demonstrated by physical and microchemical means the presence of a limiting membrane in both the side and end walls of a large number of ferns. HALFT'S work was verified in the following year by Miss BANCROFT (1), then a research scholar in the University College of Nottingham. Judging from her very lucid paper and my own results with members of this group, I should think that HALFT had shown the "real" nature of the elements in the ferns. Comprehensive as is his work, however, his statement is more so, for no mention is made of a study of any member of the Ophioglossaceae. Miss BANCROFT, also, in corroborating his work, omits this family.

Fig. 16, a drawing from the rhizome of *Ophioglossum vulgatum*, shows the typical membrane in that form. In sections stained with silver nitrate and ammonia and counterstained with methylene blue, the open scalariform pits, with their narrow, pale greenish-blue borders, are traversed by a uniform pale brown membrane. Fig. 17 illustrates the same condition in the root. Here haematoxylin accentuates the broad primary wall within the spools, and stains only faintly the membrane in the pit. The latter, indeed, often appears to be somewhat lignified, taking to a certain extent the red stain of the lignified pit borders. The petiole as it leaves the rhizome exhibits a similar type of membrane.

It was with the greatest difficulty that the membrane in *Helminthostachys* was stained sufficiently for clear demonstration. After prolonged staining with the ordinary haematoxylin and safranin solutions, it remained so vague that its presence only, but not its form, could be ascertained. The latter was finally revealed by a stain consisting of malachite green, Martius' gelb, and acid fuchsin, originally used by Dr. PIANEZE for cancer tissue. The stain was recommended by R. E. VAUGHAN (Ann. Mo. Bot. Gard. May, 1914) as a differential stain for fungus and host cells.

Fig. 18 shows the condition in the rhizome. The lignified (shaded) areas appear bright green, bounding the red of the unlignified secondary walls, which in turn bound the more deeply



stained red primary wall. In the pits, the unlignified membrane, a pale red, assumes the form of a long spindle-shaped torus. Fig. 8 is from the adult metaxylem of the root, and shows the torus lying across the space where the walls of adjacent tracheids have been torn apart in sectioning. A slight thickening was found also in the first-formed elements of the metaxylem. The tracheids

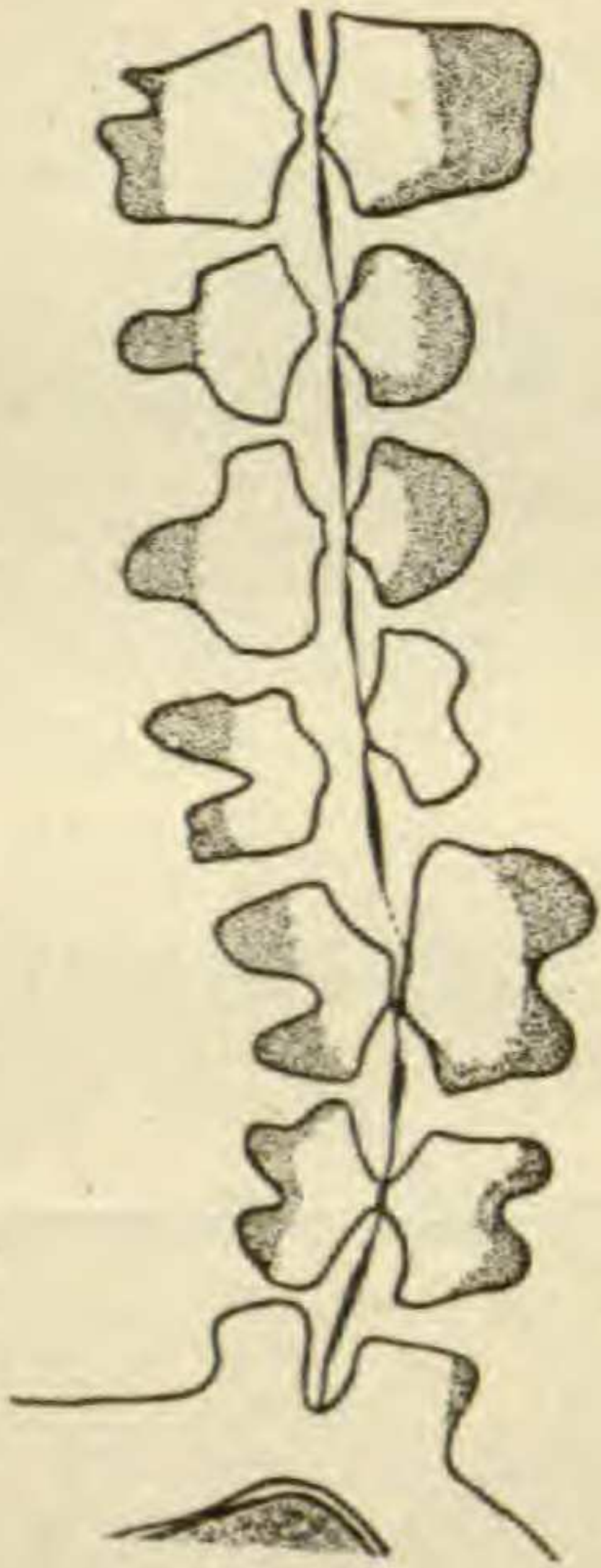


FIG. 15

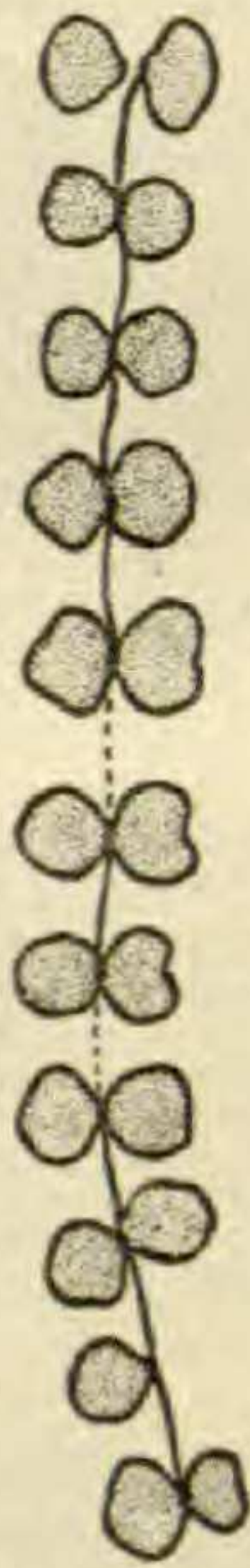


FIG. 16

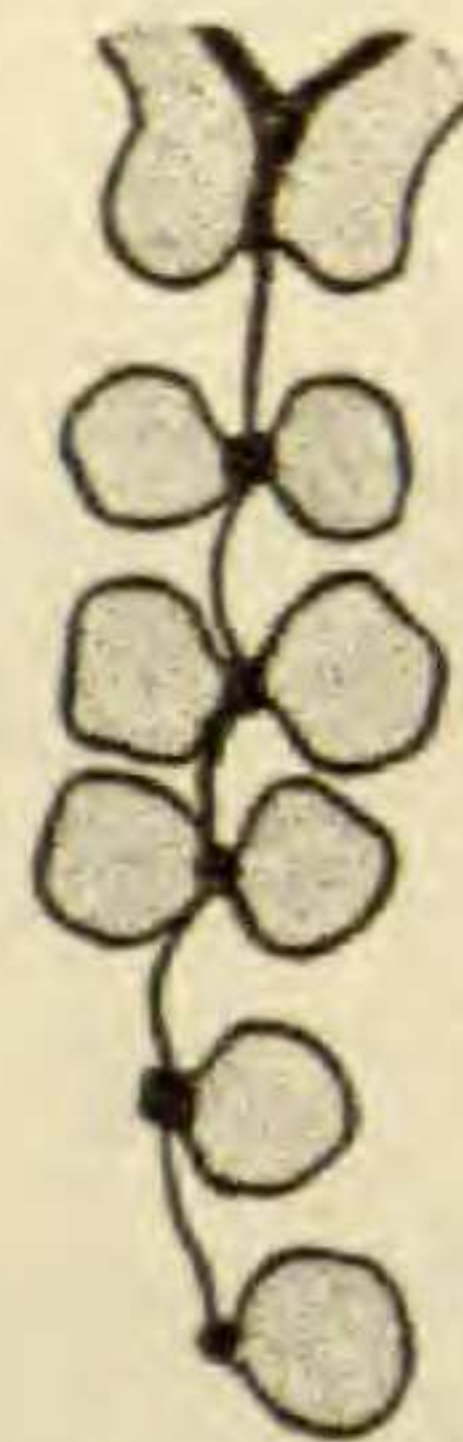


FIG. 17



FIG. 18

FIGS. 15-18.—Fig. 15, *Botrychium obliquum*: radial section of the rhizome showing pitting and torus;  $\times 1200$ ; fig. 16, *Ophioglossum vulgatum*: rhizome showing pit membrane;  $\times 900$ ; fig. 17, *Ophioglossum vulgatum*: root in cortex showing membrane;  $\times 900$ ; fig. 18, *Helminthostachys zeylanica*: longitudinal section of rhizome showing torus;  $\times 900$ .

of the petiole in longitudinal section, however, show a fine uniform membrane with only occasionally a slight thinning toward the edges of the pit.

In *Botrychium* two types of torus occur. The most common type is that seen in figs. 9 and 15, a long, slender, and rather variable spindle. This is found in the mature wood of the stem, the root, and the leaf trace in the cortex. In the last region the membrane varies from a spindle to a uniform line, as seen in transverse section in fig. 11. The arrow in a tracheid to the right of the center points to a fairly thick membrane of the uniform type.



Fig. 12 shows a number of the spindle-shaped ones at a higher magnification. The pit pores have been outlined for greater clearness. The second type of torus occurs in the immature wood of the stem and occasionally in the root. Fig. 19 shows a transverse section from the cambial region of a young rhizome of *B. obliquum*. The tracheids are only slightly lignified, some still showing the contents. Here the torus is a short oval structure as

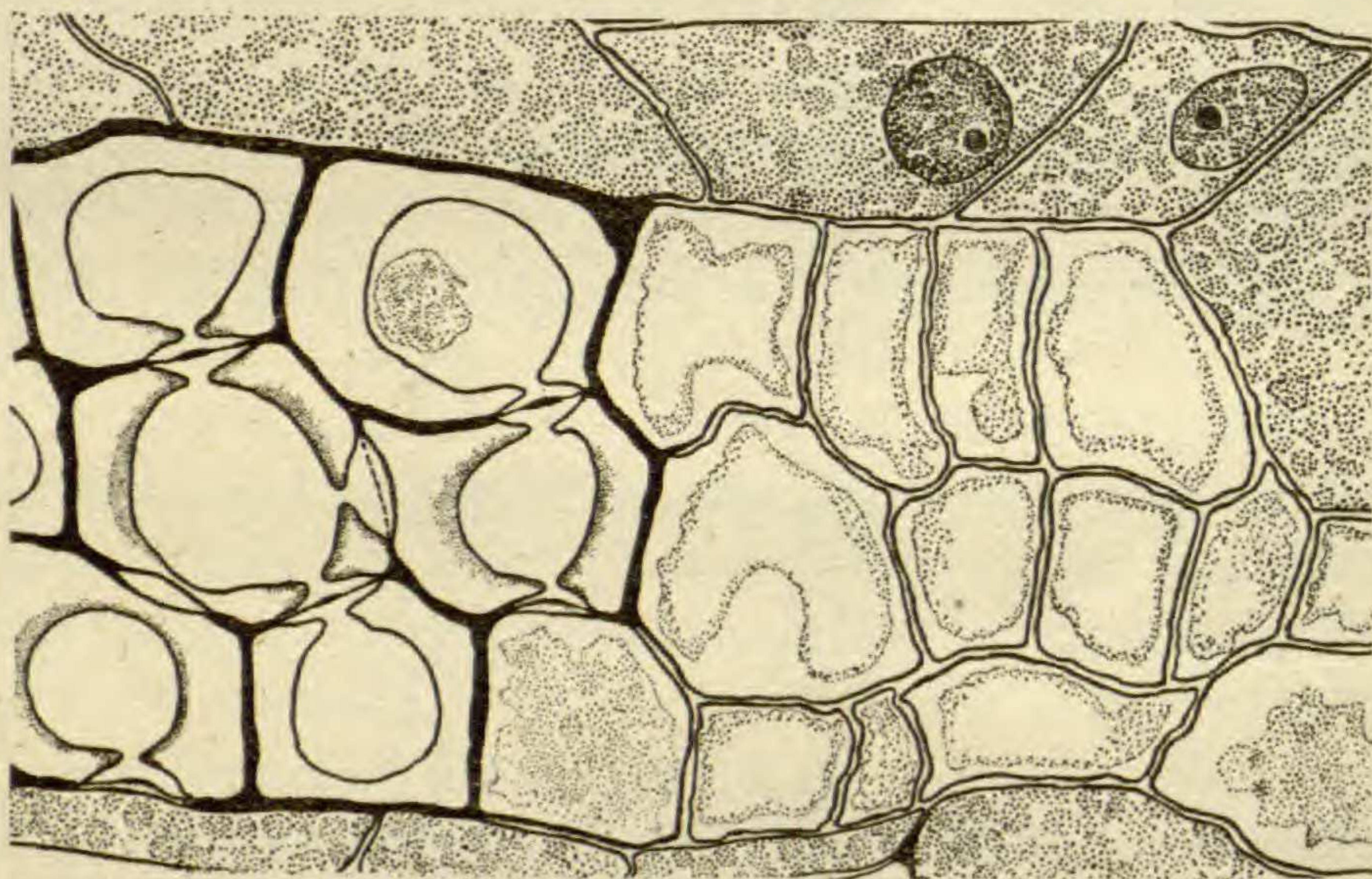


FIG. 19.—*Botrychium obliquum*: transverse section of young rhizome, at cambium showing torus and double membrane;  $\times 600$ .

long as, or slightly longer than, the pore of the pit, and connected to its edges by a fine membrane.

This section (fig. 19) also illustrates a feature which I have observed in other forms, that is, the double nature of the membrane. In the pit of the tracheid at the left-hand lower corner the membrane is of a double character. The tracheid lies against a parenchyma cell of the ray, and only the half of the membrane next to the wood cell has been thickened, while that lying next to the ray cell remains uniform. The same double nature and plano-convex thickening of the membrane are shown in the third cell to the right. Here a tracheid, as yet unlignified and filled with contents, is adjacent to one which is more advanced in development, and the thickening occurs only on the side of the latter.



A peculiar condition is occasionally met with in the stem. The tracheids are more or less discolored when cut, and stain in a peculiar manner. With Pianezze's stain the membrane becomes yellow. It is usually uniform in thickness, but swollen, occasionally almost entirely filling the pit (fig. 13).

In the petiole of *Botrychium*, as in that of *Helminthostachys*, a uniform membrane prevails. With the exception of the petiole, therefore, and peculiar unnatural spots in the stem, the typical pit membrane in *Botrychium* has a torus.

Thus the only torus I have found among the cryptogams occurs in forms whose pits are broad-bordered and circular or oval in shape. STRASBURGER (8) makes the statement that a torus occurs in *Pteris aquilina*, but he neither enlarges on the statement nor illustrates it. DEBARY (3) describes and pictures for *Pteris* an almost imperceptible one-sided swelling of the membrane, lying to one side of the pit and acting, he states, as a lid to the pit pore. I have searched in vain for such a torus. Frequently the membrane may have a "kink" toward the pit pore simulating the appearance of a torus, but both its edges follow the curve to an equal extent, thus precluding the possibility of a thickening at that point. In *Pteris* the membrane in the pits between tracheid and tracheid invariably remains uniform in thickness. As has been shown in *Botrychium*, a plano-convex torus such as DEBARY describes may occur in the pits of a tracheid where it touches a ray cell. In *Pteris*, however, the membrane even in this region remains consistently uniform. *Equisetum*, *Psilotum*, and *Isoetes*, forms with narrow-bordered pits of the scalariform type, and a number of ferns (including *Ophioglossum*), with the same type of pitting, all show a definitely uniform membrane. In *Helminthostachys* and *Botrychium*, whose pits are circular, broad-bordered, and round-pored, there is developed a definite torus. Although this suggests a possible relation of the torus to the form of the pit, the question of its relationship, whether structural, ecological, or phylogenetic, is one on which it is hoped more light may be thrown after a study of the nature and occurrence of the torus in the other groups of the plant kingdom. It is interesting to note, however, that the form of the torus in *Botrychium*



and *Helminthostachys*, whose pitting is strikingly similar to that of the seed plants, resembles closely the type I have found in the lower gymnosperms, in *Ginkgo* and the araucarians, forms which are to be described later.

To Professor R. B. THOMSON, under whose direction this work has been carried on, is due my grateful acknowledgment of his invaluable assistance and advice. I am indebted also to both Professor THOMSON and Professor J. H. FAULL for material, some of which was obtained originally through the kindness of the Director of the Royal Botanic Gardens, Kew.

UNIVERSITY OF TORONTO

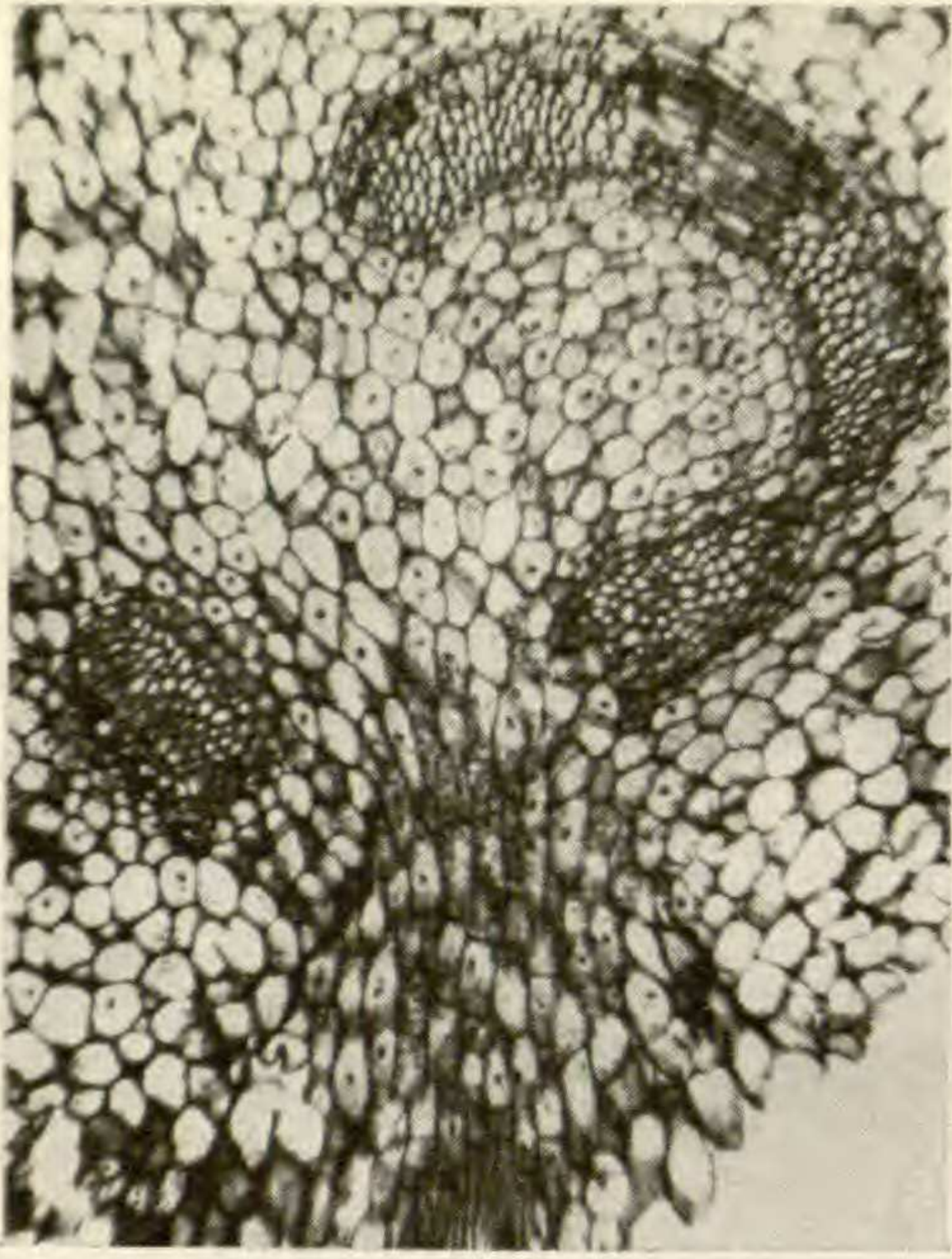
#### LITERATURE CITED

1. BANCROFT, N., On the xylem elements of the Pteridophyta. *Ann. Botany* 25:745-758. 1911.
2. BOODLE, L. A., On some points in the anatomy of the Ophioglossaceae. *Ann. Botany* 13:377-394. 1899.
3. DEBARY, A., Comparative anatomy of the Phanerogams and Ferns, pp. 161-162. 1884.
4. FARMER, J. B., and FREEMAN, W. G., On the structure and affinities of *Helminthostachys zeylanica*. *Ann. Botany* 13:421-445. 1899.
5. GWYNNE-VAUGHAN, D. T., On the real nature of the tracheae in ferns. *Ann. Botany* 22:517-523. 1908.
6. HALFT, F., Die Schlieszhaut der Hoftüpfel im Xylem der Gefäßkryptogamen. Dissertation. 1910.
7. RUSSOW, E., Vergleichende Untersuchungen. *Mem. Acad. Imp. Sci. Saint Petersburg* 19:1-207. 1872.
8. STRASBURGER, E., *Das Botanische Practicum*, p. 249. 1897.

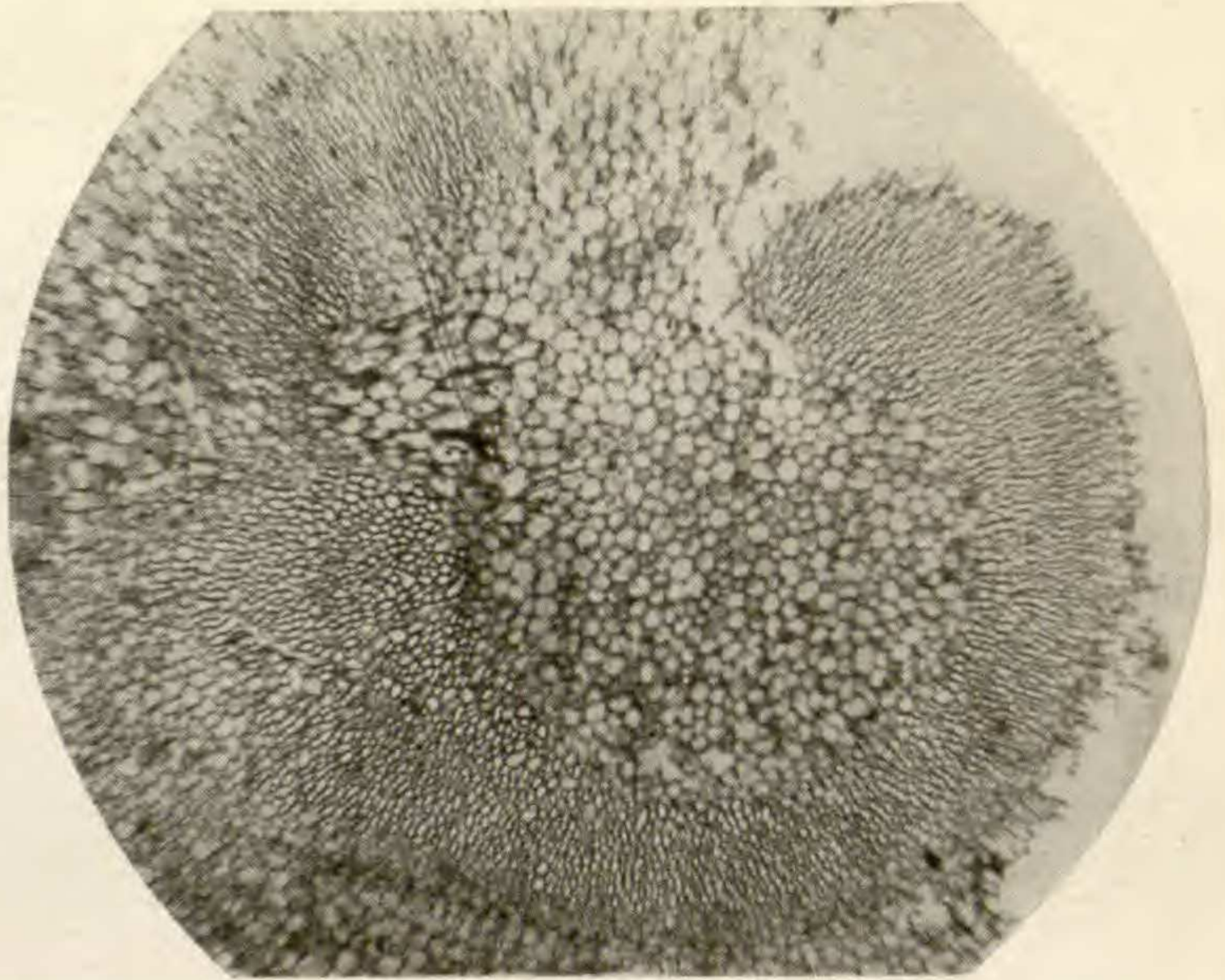
#### EXPLANATION OF PLATES XI, XII

- FIG. 1.—*Ophioglossum vulgatum*: transverse section of rhizome;  $\times 50$ .  
 FIG. 2.—*Helminthostachys zeylanica*: transverse section of rhizome;  $\times 35$ .  
 FIG. 3.—*Botrychium virginianum*: transverse section of rhizome;  $\times 40$ .  
 FIG. 4.—*Ophioglossum vulgatum*: root, transverse section;  $\times 210$ .  
 FIG. 5.—*Botrychium virginianum*: root, transverse section;  $\times 120$ .  
 FIG. 6.—*Helminthostachys zeylanica*: root, transverse section;  $\times 140$ .  
 FIG. 7.—*Ophioglossum vulgatum*: metaxylem of root showing pitting;  $\times 875$ .

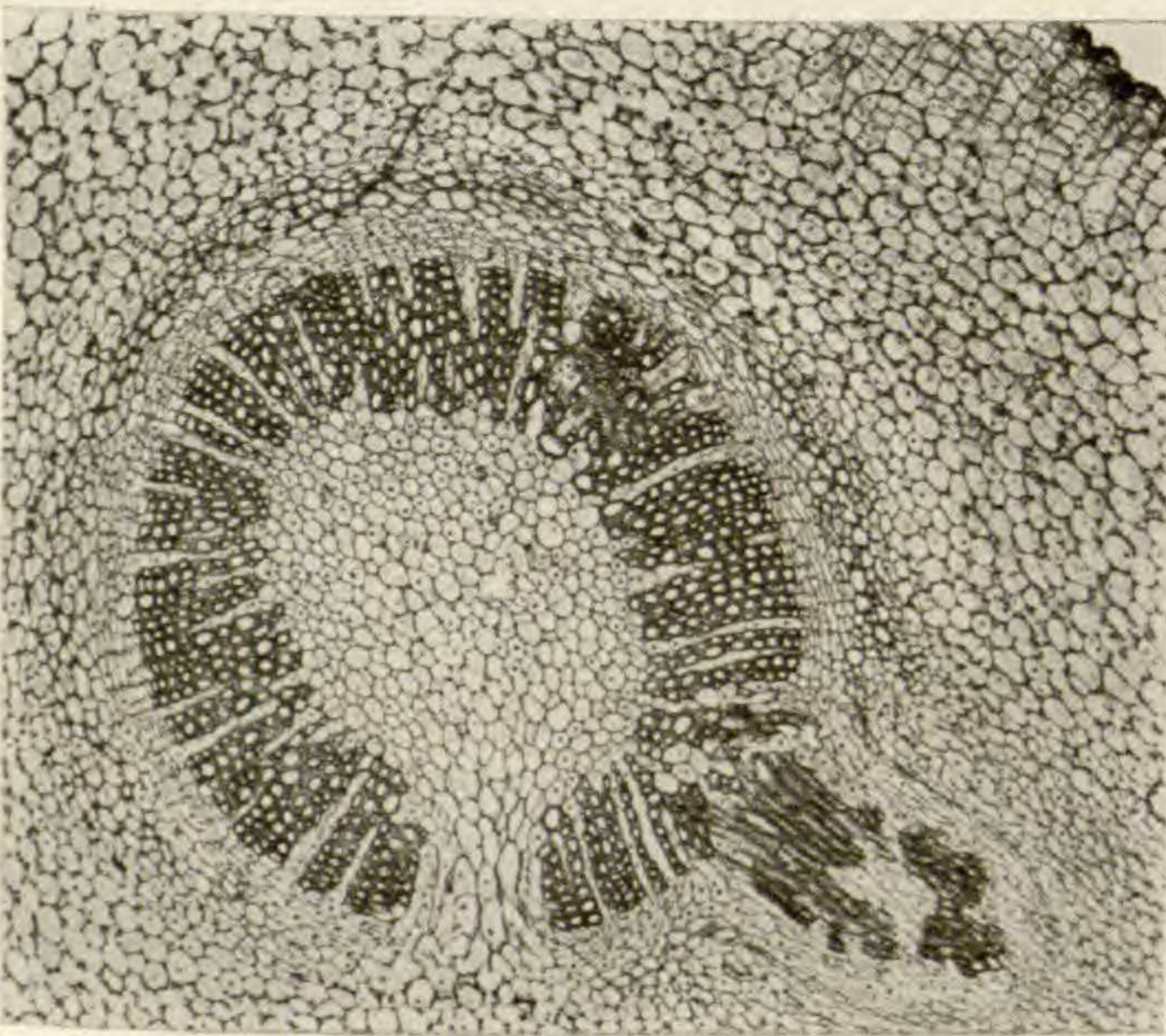




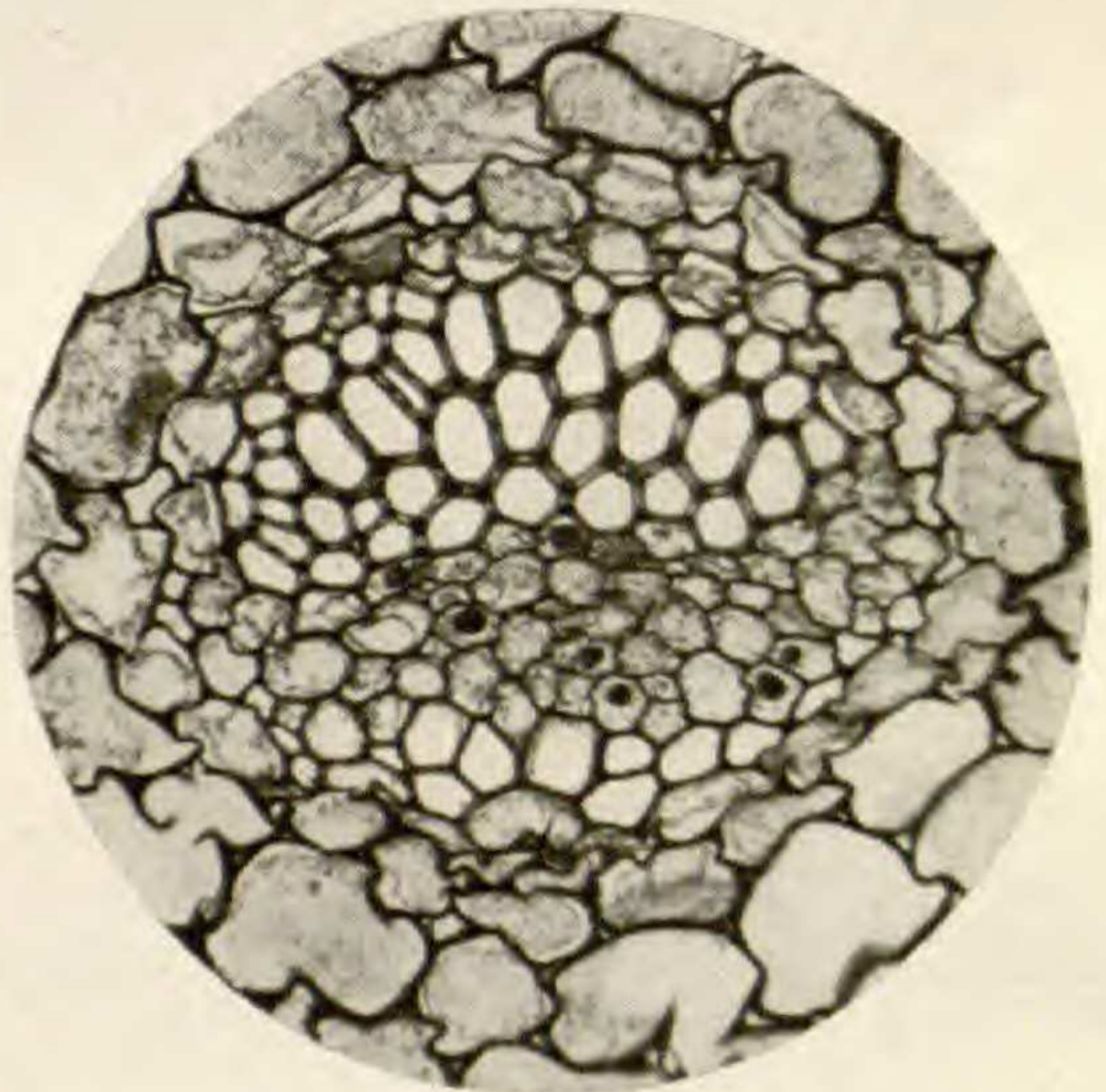
1



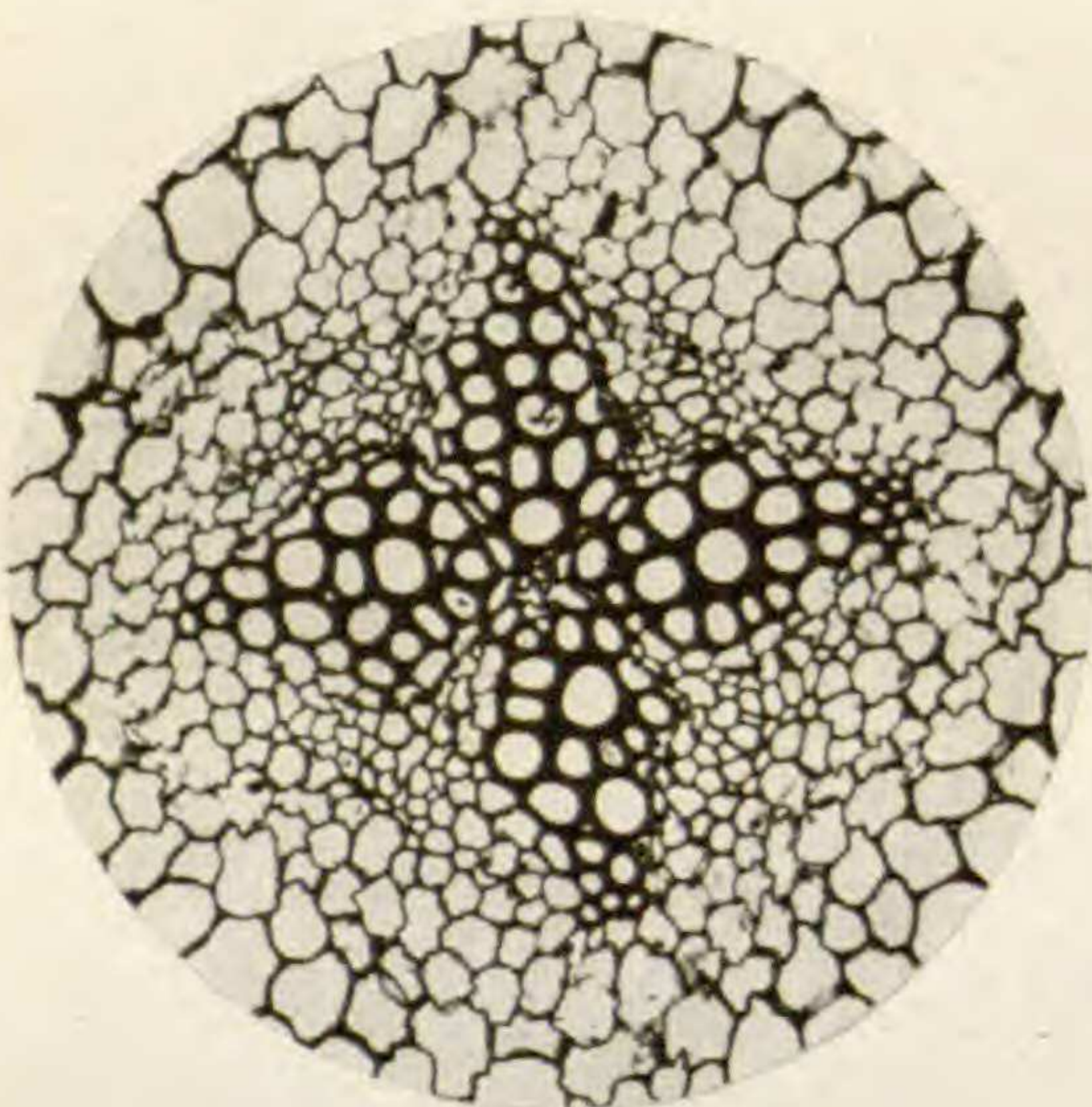
2



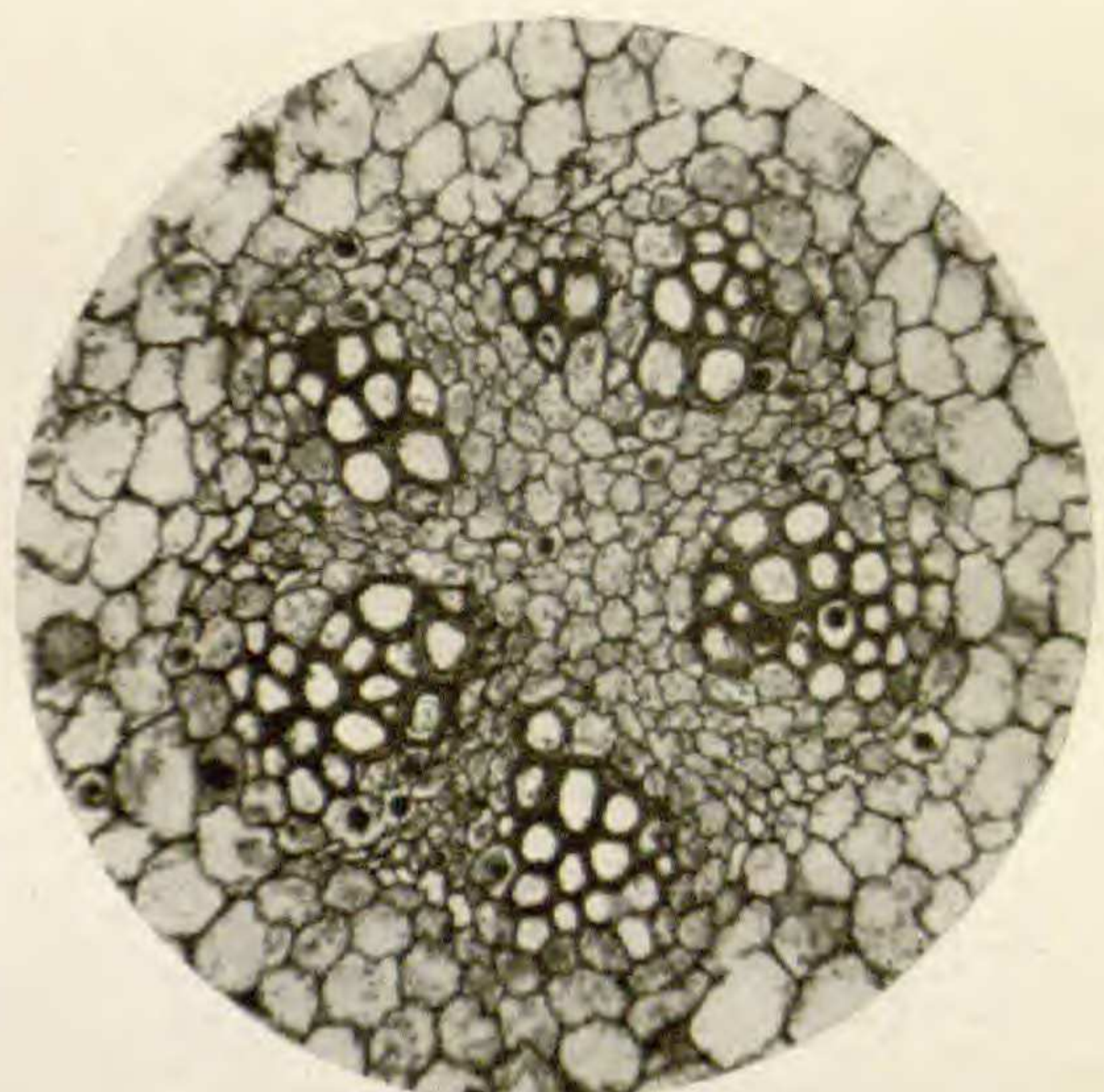
3



4



5



6